UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 0 6 2016

MEMORANDUM

SUBJECT: Review of the Response from Valent Biosciences Corp., XenTari® *Bacillus thuringiensis* subsp. *aizawai* request ABTS-1857 for label amendment, Decision # 512930

FROM: Milutin S. Djurickovic, M.S., Biologist
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TO: Susanne Cerrelli, Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
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Thru: Shannon Borges, Senior Scientist
Microbial Pesticides Branch, Biopesticides and
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ACTION REQUESTED: Review of the Response from Valent Biosciences Corp., XenTari® *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 request for label Amendment Justification for the elimination of Aerial Application restriction on Rice and Cereal Crops, Decision # 512930, Submission # 993937, DP # 436550

CONCLUSION: Due to the low EEC's expected from only 2 applications total per year on flooded grain and rice fields and lack of anticipated toxicity for aquatic organisms given the low EECs, EPA concludes that the addition of teff to the label and aerial spraying of flooded rice fields at a maximum of 2 applications of 2 lbs/acre per crop per year, with a required flood water holding period of 7 days, will not result in adverse effects to aquatic nontarget organisms.

DATA REVIEW RECORD

Active Ingredient: *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857
Product Name: XenTari®
Company Name: Valent Biosciences Corp.
DP Barcode: 436550
Decision No. : 512930
Submission No. : 993937
MRID's: 50090901-50090902

BACKGROUND:

EPA previously completed a review of XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 on June 13, 2016[1]. This review was revised on July 12, 2016[2] to delete a statement that was incorrect and to include further discuss aerial spraying to flooded grain crops and rice. The revised memo also requested that the registrant justify seven days as the maximum holding periodfor flood waters, when considering several applications, to allow for the degradation of *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857.

EPA also previously concluded that adverse effects to aquatic organisms are not expected as a result of the addition of teff to the current label. However, potential risk for aquatic organisms was identified for aerial spraying of XenTari® *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 on flooded teff (and other cereal grains cropped before Rice) or flooded rice beds due to the potential persistence of *Bt aizawai* spores and toxins in soil and aquatic ecosystems, and data indicating the potential negative impact on aquatic invertebrates and fish. EPA suggested that the registrant should specifically justify why 7 days is the maximum time to hold floodwaters.

EPA and Valent Biosciences Corp. discussed the deficiencies during a conference call on July 21, 2016, and Valent Biosciences Corp. submitted a rationale on October 12, 2016, contained in MRID 50090901, with justification for the elimination of the aerial application restriction of XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 on flooded rice beds. Additionally, Valent Biosciences Corp. submitted a new label and a SDS-PAGE study MRID 50090902 to quantify the amount of protoxins in the technical powder to support the argument that the expected environmental concentration was lower than that calculated by EPA.

Discussion

In MRID 50090901, Valent Biosciences Corp. addressed the deficiencies EPA outlined in the July 12, 2016[2] memo. In EPA's July 12, 2016[2] memo and during the July conference call, EPA had requested that the registrant discuss the 7 day holding period for floodwaters with technical field personnel. Technical field personnel from Valent have stated that the standard holding period in conventional farming practices is 7 days and that an equivalent holding period is necessary to match standard practices. Field personnel have also recommended that no more than 2 applications occur in a growing season, and the new label reflects these changes.

Valent has also proposed a modification to the EEC calculation presented by EPA. The original EEC calculations are as follows:

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[1] Memorandum from Milutin S. Djurickovic thru Shannon Borges to Susanne Cerrelli, Subject: Review of the Valent Biosciences Corp., XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 for label amendment, Decision #512930, dated June 13, 2016.

[2] Memorandum from Milutin S. Djurickovic thru Shannon Borges to Susanne Cerrelli, Subject: Revised Review of the Valent Biosciences Corp., XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 for label amendment, Decision #512930, dated July 12, 2016.

Calculation of EEC #1[3]

EEC (1.08 lbs TGAI/acre)(1 acre/0.4047 ha)(454g/lb)(10 ha)=12115.64 g
12115.64 g (.10) = **1211.56 g**. 12115.64 g (.05) = **605.78 g**
1211.56 g + 605.78 g = 1817.34 g
(1817.34 g/1 ha)(1/2 m)(1 ha/10,000 m^2)(1000 cm^3/1 L)(1 m^3/1,000,000 cm^3)(1000 mg/1 g)
= **0.091 mg/L**

Calculation of EEC #2[4]

(1.08 lbs TGAI/acre)(454,000 mg/1 lb)(1 acre/40,468,564 cm^2)(1/10 cm)(1000 mL/1 L)
= **1.21 mg/L**

Valent outlined in MRID 50090902 SDS-PAGE Protein Analysis of XenTari® technical powder, which is considered acceptable by EPA, that the amount of protoxin in XenTari® is between 4.5 and 5.3% which would decrease the maximum EEC for direct application to water from 1.21 mg/L to 0.238 mg/L at a maximum of two applications per growing season. However, the potential presence of a heat labile exotoxin that is most likely responsible for the toxicity to *Daphnia*, as concluded by EPA in a previous risk assessment (see below), is not a direct result of the active ingredient, which indicates that it is more than a protoxin that is responsible for toxicity to *Daphnia*. Therefore, the original EEC's calculated by EPA will continue to be used for EEC estimations.

In the 1997 memo Freshwater Aquatic Organism Risk Assessment for XenTari[5] (which discusses MRID's 43757601, 43828801, and 42819101) the conclusion stated that the technical powder was slightly toxic to *Daphnia* (data analyzed from MRID 43757601) and practically nontoxic to Rainbow trout. However, the trout study MRID 43828801 was rated "Supplemental, but upgradeable" due to discrepancies in the treatment vessel for fish in the test groups. Valent did not have a copy of this risk assessment, and EPA provided the 1997 EPA memo[5] to Valent after the July 20, 2016 conference call. In the 1997 EPA memo[5], mortality for *Daphnia* was recorded at higher treatments including 10, 20, and 34 mg/L (data analyzed from MRID 43757601). Turbidity was noted at the two highest concentrations where mortality at day 10 was highest (95 and 100%). At 10 mg/L mortality was 30% at day 10 with no turbidity. Other concentrations ranging from 1.1 to 5.9 mg/L had no mortality and no turbidity was observed. The 10 day LC_{50} was determined to be 12 mg/L and the 48 hour LC_{50} was determined to be greater than the highest dose tested of 34 mg/L. On pg. 3 of the 1997 memo[5], EPA concluded that *Daphnia* mortality was caused by a heat labile exotoxin produced during fermentation that complexed to the spore crystal during spray drying of the primary powder. Valent has stated that this

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[3] This EEC was estimated based on the GENEEC model, which assumed 10% and 5% would reach adjacent surface water due to runoff and drift, respectively. The GENEEC model calculated the amount of a pesticide that would reach a 1 ha body of water 2 m deep via runoff and drift from a surrounding treated area of 10 ha

[4] Based on an assumption of direct application to a 10 cm body of water, consistent with assumptions of EPA's Tier I Rice Model.

[5] Memorandum from M. Mendelsohn through Z. Vaituzis to P. Hutton. Subject: Freshwater aquatic organism risk assessment and revision of predatory wasp environmental hazards statement for XenTari (*Bacillus thuringiensis* subspecies *aizawai*), dated November 1997.

statement originally came from a review by EPA of MRID 41974802, dated June 14, 1993, which is an acute daphnid study. EPA previously reviewed the study April, 1, 1991 MRID 41974802 as discussed in EPA 1992 to 1995 memos[6] and determined that survival in the control, 0.5 mg/L, and 5 mg/L test groups was mostly 100% and in a few replicates 80% up to 21 days. At the highest level tested (74 mg/L) survival was low, and no sublethal effects were observed. This study was conducted as a limit test to establish ranges for further testing if deemed necessary. The main conclusion from the memos and DER's related to MRID 41974802 completed by EPA from 1992 to 1995[6] reiterate that toxicity to *Daphnia* is due to either the spore-crystal complex or a heat labile exotoxin. EPA suggested in those memos that the applicant perform a study that test *Daphnia* toxicity at a series of doses, which the applicant provided in MRID 43828801. The applicant also submitted a new manufacturing process in 1995, and EPA concluded in the 1995 memo[7] that this new manufacturing process will not increase toxicity to freshwater aquatic organisms. As previously stated, the 1997 memo[5] (Freshwater Aquatic Organism Risk Assessment for XenTari) concluded that toxicity to *Daphnia* was slightly toxic.

Valent is currently rebutting EPA's conclusion that toxicity is due to a heat labile exotoxin, and has stated that toxicity was most likely due to turbidity in the initial range finding study MRID 41974802. While concentrations were much higher in the original study than in the later study (MRID 43757601), which had a series of doses (1.9, 3.2, 5.4, 9, 15, 25, 42 mg/L), the later study showed that there is not enough evidence to conclude that turbidity alone caused the daphnid mortalities since mortality occurred in treatments with no turbidity at 15 mg/L. An additional study submitted on *Daphnia* MRID 42819101 reviewed in the 1997 memo[5] also concluded that toxicity was due to a heat labile exotoxin.

In summary, EPA has reviewed four studies and all the previous EPA risk assessments to support this label amendment submission and has concluded that Valent's rebuttal does not change EPA's previous conclusion regarding the heat labile exotoxin, due to the presence of daphnid mortality in treatments with no turbidity.

Additionally, in response to EPA's request, Valent has provided a discussion regarding literature references that show longer half-lives and persistence of Cry toxins in the environment. Valent and EPA now agree that the literature supports the claim that half-lives of Cry toxins produced by *Bt aizawai* range between 2.7 to 10 days, and that persistence of these Cry toxins in soil is greater than in water, with the exception that persistence in water increases in the presence of

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[6] EPA EEB Memo Submission # 401312 Mike Mendelsohn/Phil Hutton. Centari – Bacillus Thuringiensis Berliner subsp. aizawai (ABG-6305) Strain ABTS-1857 July 30, 1992.
EPA EFED Memo MRID 42819101. Clayton Beagle, Robert Rose, Elizabeth Leovey to Phillip Hutton and Michael Mendelsohn. Acute Toxicity of components of ABG-6305 to the daphnid. *Daphnia magna* – Addendum to MRID No. 41974802. July 20, 1994
EPA EFED Memo MRID 42909901. Clayton Beagle, Robert Rose, Elizabeth Leovey to Phillip Hutton and Michael Mendelsohn XenTari technical powder (ABG-6305) – infectivity and pathogenicity to rainbow trout (*Onorhynchus mykiss*) during a 20 day static renewal test. July 20, 1994.
EPA Memo Clayton Beagle to Phillip Hutton. Evaluation of the results of Xentari *Daphnia* special study. January 12, 1994.

[7] EPA BPPD Memo. Clayton Beagle and Robert Rose to Michael Mendelsohn. Abbott's summary of February 8, 1995 meeting on XenTari conditional registration requirements. June 1, 1995.

vegetation (Boisvert & Boisvert 1999). However, 20% of Cry toxins can still be present after 28 days in the field (Hung, Truong et al. 2016). Due to the limitation of a maximum of only two applications per growing season, this would keep the EEC's fairly low at 0.182 mg/L for runoff and 2.42 mg/L[8] for direct application to water. These concentrations are not expected to be toxic to *Daphnia* because at 1.1 to 5.9 mg/L no toxicity was observed as shown in the study MRID 43757601 which has been reviewed in the 1997 memo[5] . With only 2 applications per year, the Cry toxins are expected to degrade to background levels prior to the next growing season and negative effects on aquatic organisms are not expected.

References

Boisvert, M. and J. Boisvert. 1999. Persistence of toxic activity and recycling of Bacillus thuringiensis var. israelensis in cold water: field experiments using diffusion chambers in a pond. Biocontrol Science and Technology 9: 507-522.

Hung, T.P., L.V. Truong, N.D. Binh, R. Frutos, H. Quiquampoix and S. Staunton. 2016. Fate of insecticidal *Bacillus thuringiensis* Cry protein in soil: differences between purified toxin and biopesticide formulation. Pest management science.

[8] These EEC calculations are simply twice the calculated EEC's shown above in the body of the memo on pg.3 which reflect the two maximum applications allowed per year of XenTari on the new label.

DATA EVALUATION RECORD

EPA Primary Reviewer: Milutin S. Djurickovic, M.S., Biologist
EPA Peer Reviewer: Shannon Borges, M.S., Senior Scientist

STUDY TYPE: Protein Analysis
MRID NO: 50090902
DECISION NO: 512930
TEST MATERIAL: *Bacillus thuringiensis* subsp. *aizawai* ABTS 1857
PROJECT STUDY NO: Bta1857-VBCLOG0563
SPONSOR: Valent Biosciences Corporation
TESTING FACILITY: Valent Biosciences Corporation, 870 Technology Way Libertyville, IL 60048
TITLE OF REPORT: SDS-PAGE Protein Analysis of XenTari Technical Powder Samples (Bta strain ABTS-1857)
AUTHOR: Terry Benson
STUDY COMPLETED: January 24, 2012
CONFIDENTIALITY CLAIMS: None
GOOD LABORATORY PRACTICE: Non-GLP
CONCLUSION: Five different lots of *Bacillus thuringiensis* subsp. *aizawai* ABTS 1857 technical powder range from 4.5% to 5.3% (w/w) in protoxin levels.
CLASSIFICATION: Acceptable

Test Material

Bacillus thuringiensis subsp. *aizawai* ABTS 1857 technical powder

Test Methods

From five lots of the technical powder 25 mg of the test substance technical powder were separated into 1.2 ml microfuge tubes. Each sample was treated with 1 mL of NaCL, 5 mM EDTA pII8.0, and was vortexed and resuspended and centrifuged. The supernatant was poured off, and the pellet was washed again with 1 mL of 5mM EDTA at a pH of 8. The pellet was centrifuged for 1 minute and the supernatant was removed without disturbing the pellet. The sample was resuspended in 500 uL of 5mM EDTA pH 8, using Pellet Pestle. Another 500 uL of 5mM EDTA pH 8 was added to bring the volume to 1 mL and was then vortexed. 80 uL of the sample was transferred into a new 1.2 mL microfuge tube and centrifuged. The remaining liquid was then removed from the pellet before being resuspended in 200 uL of Sample Buffer "C" pH 10. The sample was boiled for 10 minutes and 200 uL of Sample buffer "D" pH 8.8 was added. The sample was mixed and centrifuged prior to loading onto duplicate 4-15% Tris-glycine gels with a molecular marker on the gel being loaded.

A Btk technical powder known to have a protoxin was prepared and used as the control. The gels were electrophoresed for 2 hours at 125 V in 25mM Tris base, 192 mM glycine, 0.1% SDS, pH 8.3, were fixed in 70% methanol and 10% acetic acid for 30 minutes, and stained using Coomassie G-250 for 1 hour with agitation. The gels were destained for 30 minutes and were analyzed using a GS-710 densitometer and Quantity One 4.6.9 software. Lane based quantitation

with background subtraction was done. The values from the standard lanes were used to generate a regression line to determine the protoxin values. The Cry1A and Cry2A bands were analyzed independently and added together to give protoxin total.

Results Summary

Xentari TP Sample Lot Number	% Total Protoxin in sample (w/w)	%CV
207-160-V900	5.3	6.6
208-162-V900	4.5	4.1
208-163-V900	4.8	5.6
208-164-V900	4.9	9.3
208-165-V900	4.8	4.4

Conclusions

Five different lots of *Bacillus thuringiensis* subsp. *aizawai* ABTS 1857 technical powder range from 4.5% to 5.3% (w/w) in protoxin levels.